Liparis purpureovittata (Orchidaceae)—a New Species from Japan

CHIE TSUTSUMI, TOMOHISA YUKAWA and MASAHIRO KATO

Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan

A new species, *Liparis purpureovittata* (Orchidaceae), is described from central and northern Japan on the basis of both morphological and molecular characters. This species is distinguishable from the closely related *Liparis kumokiri* in the abruptly dilated labellum (vs. the gradually dilated labellum in *L. kumokiri*), the labellum recurved strongly in the middle (vs. the labellum strongly recurved in both the middle and distal part), and the column with rounded marginal wings (vs. angular marginal column wings).

Key words: Japan, Liparis purpureovittata, new species, Orchidaceae

Liparis L. C. Rich. (Orchidaceae) is a cosmopolitan genus comprising ca. 420 species (Cribb & Govaerts 2005). Section Liparis, one of 19 sections in the genus, is characterized by a small, undeveloped pseudobulb at flowering time and subfleshy, non-ribbed leaves arising from the apex of the pseudobulb (Garay & Gonzalez 1999). In Japan, eight species of sect. Liparis are recognized, but several materials do not coincide with the described species (Takahashi 1987, Hashimoto 1990). One of them, related to L. kumokiri F. Maek., has been called "Azumi-kumokiri," "Chikuma-jigabachi," "Fugaku-kumokiri" or "Nambu-kumokiri" (Takahashi 1985, 1987, Okuhara 1992, Kurihara et al. 1993). In this paper we describe the entity as a new species, Liparis purpureovittata, and discuss its morphological and molecular characters.

Materials and Methods

A previous molecular phylogenetic study of *Liparis* sect. *Liparis* (Tsutsumi *et al.* 2007), which

suggested a specific status for this entity, examined two samples from Hokkaido (No. 24) and a cultivated stock of uncertain origin (No. 27) for it. In this study, we analyzed DNA sequences of two additional samples of the same plant from Niigata Pref., Minami-uonuma-gun, Yuzawa-cho (C. Tsutsumi, T. Kuhara & M. Sato CT1089), and from Nagano Pref., Saku, Mt. Tateshina (M. Matsui CT1145). Procedures for extraction, amplification and sequencing of DNA followed those described in Tsutsumi et al. (2007). The regions we examined were the internal transcribed spacer regions of the 18S-26S nuclear ribosomal DNA (ITS) and three plastid regions, trnL with trnL-trnF spacer, trnStrnG spacer and a part of the maturase-encoding gene (matK) (GenBank accession Nos. of ITS, trnL with trnL-trnF spacer, trnS-trnG spacer, and matK are AB366610, AB366611, AB366612, AB366613 for the Niigata sample, and AB366614, AB366615, AB366616, AB366617 for the Nagano sample). Primers for PCR amplification and sequencing were the same as those in Tsutsumi et al. (2007).

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Vouchers are deposited in the Herbarium of the National Museum of Nature and Science (TNS).

Taxonomy

Liparis purpureovittata Tsutsumi, T. Yukawa & M. Kato, **sp. nov**. (Figs. 1, 2)

Differt a *L. kumokiri* labello viridi, supra basin abrupte dilatato, sulco medio et labello basi atropurpureo; a *L. koreana* et *L. fujisanensi* sepalis lateralibus latis revolutisque.

Typus. JAPAN, Honshu: Niigata Pref., Minamiuonuma-gun, Yuzawa-cho, alt. ca. 1340 m, terrestrial on damp ground under bushes, 17 July 2006, *C. Tsutsumi, T. Kuhara & M. Sato CT1089* (holo-TNS)

Pseudobulb ovoid, 1-2 cm long. Leaves 2, ovateelliptic, margin entire or somewhat undulate, obtuse or subacute, 5-13 cm long, 2-5 cm wide, conduplicate, glossy, glabrous, green; petiole 2-6 cm long, winged, nearly as long as blade. Inflorescence terminal, racemose, 10-25 cm long, bearing 4-14 flowers; axis glabrous, ridged, green. Bract ovate, acute, 2-5 mm long, green. Pedicellate ovary clavate, twisted, 8-11 mm long, green, sometimes with purplish tint at base. Dorsal sepal linearlanceolate, subacute, occasionally slightly revolute, erect or somewhat recurved, 8-9 mm long, 2-2.5 mm broad, greenish purplish. Lateral sepals obliquely ovate or obliquely lanceolate, subacute, revolute, distally twisted, partially enfolding lip, 7-9 mm long, 3-3.5 mm broad, greenish purplish. Petals falcate, linear, obtuse, strongly revolute, pendulous, sometimes slightly twisted, 8-9 mm long, 0.5 mm broad, purplish. Labellum entire or minutely erose, ovate-oblong, shortly clawed, strongly recurved at middle, margins sometimes slightly



Fig. 1. Liparis purpureovittata Tsutsumi, T. Yukawa & M. Kato at the type locality, Yuzawa-cho, Niigata Pref., Japan (A-C). A: Habit. B: Inflorescence. C: Flower, front view. Photographs taken by C. Tsutsumi in A, and by K. Suzuki in B and C.

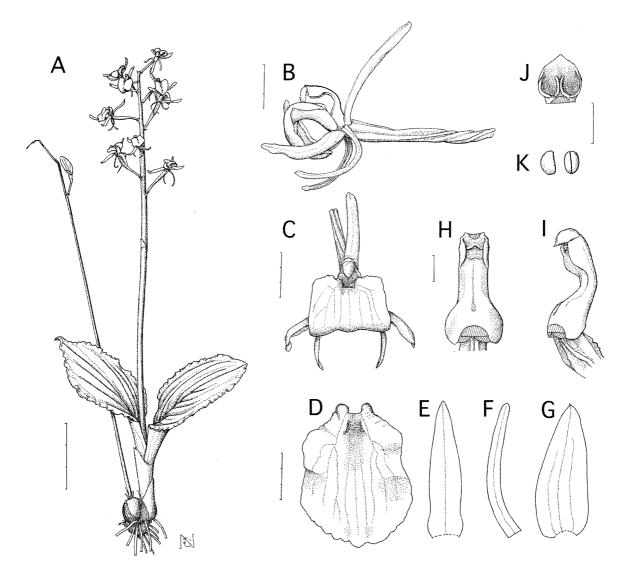


FIG. 2. Liparis purpureovittata. A: Habit. B: Flower, side view. C: Flower, front view. D: Labellum. E: Dorsal sepal. F: Petal. G: Lateral sepal. H: Column from below. I: Column, side view. J: Anther cap. K: Pollinia. Drawn by M. Nakajima from the holotype (C. Tsutsumi, T. Kuhara & M. Sato CT1089). Scale bars = 3 cm (A), 3 mm (B-G) or 1 mm (H-K).

revolute, base subtruncate and abruptly dilated above the base, obtuse or apiculate, 8-9 mm long, 6-7 mm broad, dull yellowish green, base and central groove light to dark purple. *Column* terete, incurved, apically with rounded wings, base dilated, 4-5 mm long, ventral surface pale green, other parts green; pollinia 4, in 2 pairs, waxy, yellow; anther cap ovoid, mucronate, green.

Distribution. JAPAN: Hokkaido and central Honshu.

Japanese name. Shiten-kumokiri (nov.). The

vernacular name, from the purple mark on the lip, is proposed for *Liparis purpureovittata*. It has previously been given several provisional names based on the places where local populations were found.

Additional specimens examined. JAPAN. Hokkaido. Esashi-gun, Nakatonbetsu-cho, C. Tsutsumi L27 (TNS). Honshu. Gunma Pref.: Minakami, Mt. Tanigawa-dake, Shirakaba-goya, 2 July 1953, T. Yamazaki (TI). — Nagano Pref.: Saku, Mt. Tateshina, M. Matsui CT1145 (TNS). — Precise locality unknown (cultivated at Tsukuba Botanical Garden, National Museum of Nature and Science), C. Tsutsumi L24 (TNS).

TABLE 1. Number of substitutions (bp) in nuclear ribosomal ITS region and three plastid regions among *Liparis purpureovittata* and closely related species (*L. kumokiri*, *L. fujisanensis*, *L. koreana*, *L. makinoana*). Vouchers and GenBank accession numbers are shown in this study and Tsutsumi *et al.* (2007).

	L. purpureovittata	L. kumokiri	L. fujisanensis	L. koreana	L. makinoana
ITS (above diagonal) / matK (below diagonal)					
L. purpureovittata	_	10	12	12	15
L. kumokiri	1	_	2	2	7
L. fujisanensis	1	0	_	0	9
L. koreana	3	2	2	-	9
L. makinoana	3	2	2	4	_
trnLF (above diagonal) / trnSG (below diagonal	1)				
L. purpureovittata		6-7	7	7-8	9
L. kumokiri	2-4	_	2-3	2-4	8-9
L. fujisanensis	4-6	2-4		2-3	8
L. koreana	4-5	2-3	4-5		8-9
L. makinoana	10-11	8-9	10-11	10	_

Notes. Liparis purpureovittata is morphologically closely related to L. fujisanensis F. Maek. ex Konta & S. Matsumoto, L. kumokiri and L. koreana (Nakai) Nakai ex W. T. Lee. Liparis purpureovittata can be distinguished from L. kumokiri, however, by the following three characters; 1) the labellum is abruptly dilated above the base (gradually dilated in L. kumokiri); 2) the labellum is strongly recurved in the middle and slightly or not recurved distally (strongly recurved both at the base and apex in L. kumokiri); 3) the column wing is rounded (angular in L. kumokiri). Liparis purpureovittata can be also distinguished from L. fujisanensis and L. koreana by having wider (ca. 3 mm), strongly revolute lateral sepals. The lateral sepals of L. fujisanensis and L. koreana are narrower (ca. 2.5 mm) and not strongly revolute. Additionally, labellum color can distinguish those species: green with purplish middle groove in L. purpureovittata, purplish in L. fujisanensis and L. koreana, and uniformly pale yellowish green in L. kumokiri.

The molecular analysis showed that the two additional samples from Niigata and Nagano of *L. purpureovittata* have no substitutions from those of No. 24 and No. 27 (Tsutsumi *et al.* 2007), except

for a single double-peaked site in the nuclear ribosomal ITS regions of the Niigata material, and a single autapomorphic substitution in the *trnS-trnG* spacer of the Nagano sample. *Liparis purpureovittata* therefore shows little intraspecific variation in the sequences we examined. The sequences of *Liparis purpureovittata* are distinct from those of closely related species (Table 1).

Our molecular phylogenetic analysis revealed that *L. purpureovittata* occupies a separate, independent phylogenetic position and is sister to a clade consisting of *L. fujisanensis*, *L. koreana* and *L. kumokiri*, and together they form a sistergroup with a clade of *L. makinoana* Schltr. and *L. japonica* (Miq.) Maxim. (Tsutsumi *et al.* 2007). These phylogenetic relationships are endorsed by a character of the anther cap. The mucronate apex of the anther cap characterizes *L. fujisanensis*, *L. koreana*, *L. kumokiri* and *L. purpureovittata*, while the anther cap is beaked in *L. makinoana* and *L. japonica*.

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